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High Yolk Testosterone Transfer Is Associated with an Increased Female Metabolic Rate

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3 **High yolk testosterone transfer is associated with an increased female metabolic rate**

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19 Running title: Metabolic costs of yolk testosterone transfer

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22 **What Is Already Known:** Yolk hormones of maternal origin are important mediators or

23 prenatal maternal effects in oviparous species. Within populations, there is large variation

in yolk hormone transfer observed among females, but the mechanisms that contribute to the maintenance of this variation are poorly understood.

What This Study Adds: Unlike most previous work that has focused on potential costs for the offspring, we here show that yolk hormone transfer is associated with metabolic costs for the mother. These costs for the mother can balance beneficial effects on offspring and thereby contribute to the maintenance of variation in maternal yolk hormone transfer in animal populations.

Abstract

Yolk androgens of maternal origin are important mediators of prenatal maternal effects. Although in many species short-term benefits of exposure to high yolk androgen concentrations for the offspring have been observed, females differ substantially in the amount of androgens they transfer to their eggs. It suggests that costs for the offspring or the mother constrain the evolution of maternal hormone transfer. However, to date the nature of these costs remains poorly understood. Unlike most previous work that focused on potential costs for the offspring, we here investigated if high yolk testosterone transfer is associated with metabolic costs (i.e. a higher metabolic rate) for the mother. We show that Japanese quail (*Coturnix japonica*) females that deposit higher testosterone concentrations into their eggs have a higher resting metabolic rate (RMR). Because a higher metabolic rate is often associated with a shorter lifespan, this relationship may explain the negative association between yolk testosterone transfer and female longevity observed in the wild. Our results suggest that metabolic costs for the mother can balance the short-term benefits of yolk testosterone exposure for the offspring, thereby contributing to the maintenance of variation in maternal yolk hormone transfer in natural populations.

Keywords: maternal effects; reproductive physiology; metabolic rate; trade-off; maintenance of variation; metabolic costs; yolk hormone transfer

Introduction

Maternally transferred yolk androgens are important mediators of prenatal maternal effects in birds and other oviparous species (Schwabl 1993). Many experimental studies have shown that exposure to high androgen concentrations before birth can have beneficial short-term effects on offspring, including an enhanced begging capacity or boosted growth (reviewed in Groothuis et al. 2005b; Gil 2008). This has raised the question why not all females deposit high androgen concentrations into their eggs, and how the large between-female variation in yolk androgen deposition is maintained in natural populations.

In an attempt to answer this question, researchers have mostly focused on potential costs of prenatal exposure to high androgen concentrations for the offspring, including immunosuppression (Groothuis et al. 2005a; Tschirren et al. 2005), metabolic costs (Eising et al. 2003; Tobler et al. 2007; Nilsson et al. 2011) or sexual antagonism (Ruuskanen et al. 2012; Tschirren 2015). The results of these studies are mixed. Costs associated with yolk androgen transfer for the mother, on the other hand, have received less attention (but see Pilz et al. 2003; Gil et al. 2006), even though the recent finding that female collared flycatchers (*Ficedula albicollis*) that deposit high testosterone concentrations into their eggs live shorter (Tschirren et al. 2014) indicates that direct costs for the mother may be substantial.

A negative association between resting metabolic rate (RMR) and lifespan is the cornerstone of the rate of living hypothesis (Pearl 1928), and evidence for such a relationship has been found among, as well as within species (Ruggiero et al. 2008; but see e.g. Duarte and Speakman 2014). If testosterone transfer to the eggs is associated with

metabolic costs for the mother, this could provide a proximate explanation for why females that deposit high testosterone concentrations into their eggs live shorter. Here, we tested if high maternal testosterone transfer is associated with an increased female metabolic rate in a precocial bird, the Japanese quail (*Coturnix japonica*) to explore if metabolic costs for the mother may constrain the evolution of maternal yolk hormone deposition.

Material and Methods

Study population

The study was conducted in a population of Japanese quail (*Coturnix japonica*) maintained at the University of Zurich, Switzerland. Males and females were housed in separate outdoor aviaries (7 x 5.5 m each). For reproduction, male-female pairs (N = 40 pairs; age: 154-184 days) were transferred to cages (122 x 50 x 50 cm) within our breeding facility. Cages contained *ad libitum* food, water, grit, a source of calcium, a shelter and a sand bath. The bottom of the cages was lined with sawdust. The breeding facility was kept on a 16 : 8 h light : dark cycle at $20 \pm 3^{\circ}\text{C}$ (see Pick et al. 2016 for details).

Yolk testosterone analysis

We collected the 5th egg of each female on the day it was laid (natural clutch size: 7-14 eggs (Hoffmann 1988)), separated yolk and albumen, weighed ($\pm 0.01\text{g}$) and homogenised the yolk, and froze it at -20°C (N = 40). Yolk testosterone (yolk T) extraction and radioimmunoassay were performed following previously published

99 protocols (Okuliarová et al. 2011). In short, 100-110 mg of yolk were spiked with
100 approximately 2500 dpm of [³H]-testosterone (PerkinElmer, USA) and extracted twice
101 with a mixture of diethyl and petroleum ether. Yolk testosterone concentrations (pg / mg
102 yolk) were quantified in 10µl aliquots using [1,2,6,7-³H]-testosterone (PerkinElmer,
103 USA, specific activity 63.47 Ci / mmol) and a specific antibody generated in rabbits
104 against testosterone-3-(carboxy-methyl) oxime bovine serum albumin conjugate (Zeman
105 et al. 1986). The sensitivity of the assay was 1.62 ± 0.17 pg per tube. The mean recovery
106 rate \pm SD was $79.3 \pm 6.4\%$. The samples were analyzed in two assays. The intra- and
107 inter-assay coefficients of variation were 4.7% and 6.5%, respectively. Measured yolk
108 testosterone concentrations were log transformed before analysis.

110 *Metabolic rate*

111 Resting metabolic rate (RMR) of females (N = 40) was measured over six consecutive
112 nights (seven birds per night) in sealed plastic metabolic chambers (3.9 l, 234 x 165 x
113 165mm, Lock & Lock, Hanacobi Co. Ltd., Korea) during the birds' rest phase (6pm –
114 8:30am) in a dark room at 25-28°C, which is within the species' thermoneutral zone
115 (Ben-Hamo et al. 2010). Food was withdrawn from the cages for two hours before
116 measurements started to ensure a post-absorptive state. We measured the fractional
117 content of oxygen and carbon dioxide in the air using an eight-channel open flow
118 respirometry system (Sable Systems, Las Vegas, USA). Before each trial, the CO₂
119 analyzer was zeroed using CO₂-free air (dry nitrogen, 99.99% N₂, PanGas, Switzerland)
120 and spanned using a 1.002 % mol CO₂ mixture (balance N₂, PanGas, Switzerland). The
121 O₂ analyzer was spanned to 20.95% by flushing dry air through the system. During a

trial, external air was pumped into the chambers (seven containing a bird and one empty control chamber) at a flow rate of 1650-1700 ml / min (Dual channel bench field pump, 3.5lpm maximal flow per channel, PP-2-1; Flow Bar Mass Flow Meter FB-8-1, Sable System, USA). All gas flow connections passed through ultra-low permeability Tygon tube (internal diameter: 8 mm).

Each recording sequence lasted 45 minutes and consisted of one round of measurements of O₂, CO₂, flow rate and temperature for each of the seven chambers containing a bird. The empty control chamber was measured at the beginning and the end of each sequence. Excurrent air from the chambers was pushed through a RM-8-2 respirometry multiplexer (Sable Systems International, Las Vegas, USA) programmed to serially divert individual gas streams every five minutes. A subsample of each gas stream (250 ml / min) was pulled through a dessicant column (magnesium perchlorate, Sigma-Aldrich, USA) before being analyzed every second over a five minute period by a fuel cell O₂ analyzer and a dual wavelength infrared bench CO₂ analyzer (Foxbox, Sable System, USA). To avoid taking measurements during the drift period (i.e. warming up) of the Foxbox, it was turned on 4 hours before the first recording started. Because of residual air in the system, we excluded the first 100 seconds of each 5 min measurement. In total, we obtained 18-20 200 sec measurement sequences per bird during the course of the night.

Oxygen consumption rates (VO₂, ml / min) were determined by comparing the oxygen content of the metabolic chamber containing birds (Fe) and the empty control chamber (Fi, baseline). Baseline O₂ and CO₂ were determined by regressing all control chamber readings against time for each 45 minute sequence. VO₂ was calculated using the following equation, which corrects for flow rate (FR) and CO₂ concentration: $VO_2 = FR$

* $((\text{FiO}_2 - \text{FeO}_2) - \text{FeO}_2 * (\text{FeCO}_2 - \text{FiCO}_2)) / (1 - \text{FeO}_2)$. We determined the mean of 60 consecutive seconds of lowest VO_2 within a range of variation of $< 0.015 \text{ ml / min}$ (i.e., three-fold the standard deviation of the control chamber). RMR (W or J / sec) was estimated from VO_2 and the respiratory exchange ratio (RER; $\text{VCO}_2 / \text{VO}_2$) using the thermal equivalence data in Withers (1992). The average RER was 0.70, indicating a predominance of lipid metabolism. The birds were weighed ($\pm 0.1\text{g}$) after the RMR measurement. Metabolic rate was measured after egg collection (see above). All females were in breeding condition (i.e. egg laying) and in the same stage of the breeding cycle when RMR was measured.

All experiments conform to the relevant regulatory standards and were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (195/2010; 14/2014; 156).

Statistical analysis

We tested for an association between yolk T concentration and female RMR using a linear mixed model that included female RMR and body mass as fixed effects. In addition, we ran the same model but with the residuals of a linear regression of RMR on body mass (residual RMR) as fixed effect. Because some of the females used in this study were sisters, we included Family ID ($N = 29$ families) as a random effect in the models. Residuals of the models were normally distributed. P values were obtained by comparing two nested models, with and without the variable of interest, using likelihood ratio tests. All analyses were performed in R (R Development Core Team 2011).

Results

We observed a significant positive association between the amount of testosterone a female transfers to her eggs and her RMR ($\chi^2 = 5.496$, $P = 0.019$; Fig. 1A). Similarly, there was a positive association between yolk T and a female's residual RMR ($\chi^2 = 5.243$, $P = 0.022$; Fig. 1B). Although there was a strong positive relationship between RMR and body mass ($\chi^2 = 18.688$, $P < 0.001$), no significant association between a female's body mass and the T concentration in her eggs was observed ($\chi^2 = 0.423$, $P = 0.516$; Fig. 1C). The results were similar when analyzing yolk T content instead of yolk T concentration (yolk T content – mother RMR: $\chi^2 = 6.781$, $P = 0.009$; yolk T content – residual mother RMR: $\chi^2 = 6.566$, $P = 0.010$).

Discussion

Our study shows that the transfer of high T concentrations to the eggs is associated with an increased female metabolic rate. If the increased RMR associated with high yolk T transfer affects a female's daily energy budget, which is likely (Buchanan et al. 2001), the resulting allocation trade-off may play a key role in balancing costs and benefits of differential maternal egg provisioning, and contribute to the maintenance of variation in maternal yolk hormone transfer among females.

Currently, we can only speculate about the proximate mechanisms that underlie the observed relationship between yolk T concentrations and female metabolic rate. Components involved in the endocrine control of reproductive physiology could either directly or indirectly explain the association. For example, the production of testosterone in the theca and granulosa cells of the follicular wall (Hackl et al. 2003) or the transfer of

testosterone from the site of production to the developing yolk might be energetically demanding. Alternatively, the observed association could be indirect, mediated, for example, by circulating T levels in the mother. Indeed, experimental studies have shown that high plasma T levels can lead to an increased metabolic rate in both birds (Buchanan et al. 2001) and men (Welle et al. 1992). However, this scenario is unlikely given that in quail maternal plasma T levels and T concentrations in the eggs appear to be unrelated (Hackl et al. 2003; Okuliarová et al. 2011). Finally, behavioral differences among females may indirectly affect both yolk testosterone transfer and metabolism (van Oers et al. 2011; Bouwhuis et al. 2014). Irrespective of the exact physiological or behavioural mechanisms that link testosterone transfer to the egg and female metabolic rate, costs associated with a high metabolic rate (e.g. Ruggiero et al. 2008; Dowling and Simmons 2009), may (at least partly) explain why females that transfer high yolk T concentrations to their eggs have a shorter lifespan (Tschirren et al. 2014). Ultimately, our study highlights that to understand the evolution of maternal effects as well as their mediators, it is crucial to consider the costs and benefits for all players involved.

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288

Figure Legend

Fig. 1. Relationship between testosterone concentrations in the eggs and A) a female's resting metabolic rate (RMR), B) the residuals of a regression of female RMR on body mass (residual RMR), and C) a female's body mass (N = 40 females).

